

# Association of Factor V Leiden and Prothrombin G20210A Polymorphisms in Women with Recurrent Pregnancy Loss in Isfahan Province, Iran

## Abstract

**Background:** Maternal thrombophilia has been identified as a risk factor for recurrent pregnancy loss (RPL). The aim of this study was to investigate the association between prothrombin G20210A and factor V Leiden (FVL) polymorphisms in women with RPL and a control group of parous women in Isfahan province of Iran. **Methods:** We studied 250 women with idiopathic RPL and 116 control cases. Prothrombin and FVL different genotypes were determined using polymerase chain reaction and reverse hybridization technique. **Results:** The frequencies of heterozygous mutation prothrombin G20210A were 6% and 0.9%, respectively ( $P = 0.025$ ), in cases compared to the control group. The frequencies of homozygous mutation prothrombin G20210A were 0.4% and 0%, respectively, in cases compared to controls ( $P = 0.02$ ). The prothrombin mutation was significantly higher in cases compared to the control group (odds ratio 8.81; 95% confidence interval: 1.16–66.62). There was no significant difference between the FVL mutation and pregnancy loss. **Conclusions:** The results indicated a significant higher frequency of prothrombin G20210A in women with RPL in comparison with controls. Our data suggest that the prothrombin G20210A mutation, but not the FVL mutation, may be an unrecognized cause of RPL in our population.

**Keywords:** Abortion, factor V, mutation, thrombophilia, pregnancy, prothrombin

## Introduction

Recurrent pregnancy loss (RPL) is defined as the occurrence of three or more consecutive spontaneous abortions. Microthromboses production in placental blood vessels and placental infarctions caused by maternal thrombophilia may be associated with RPL. In these conditions, fetal death may happen due to low placental perfusion caused by maternal thrombophilia.<sup>[1,2]</sup>

Higher incidence of certain inherited thrombophilias such as factor V Leiden (FVL) G1691A and prothrombin G20210A mutations has been the focus of some studies in women with RPL.<sup>[3,4]</sup> The G20210A prothrombin gene mutation that results in high prothrombin levels involves position 20210 in the three-untranslated region of the prothrombin gene. It has been stated that the FVL and prothrombin G20210A mutations are the most common causes for inherited thrombophilia.<sup>[1,5,6]</sup> Despite the large number of studies on relationship between FVL G1691A and prothrombin G20210A mutations with RPL,

there is a lack of certainty in considering these two mutations as the cause of RPL and results from different populations demonstrate different results. Therefore, the current study aimed to assess the possible mutation of the FVL and the prothrombin G20210A mutation by reverse hybridization technique in women of Isfahan, Iran, experiencing RPL.

## Methods

### Subjects

This case–control study was performed with 250 women (25–38 years old) experiencing RPL and referred for evaluation between April 2009 and February 2016. In addition, 116 women were included in the study as the control group. This study was approved by the Ethics Committee of Isfahan University of Medical Sciences. An informed consent was obtained from each participant. Women with at least two or more consecutive pregnancy losses before the 20<sup>th</sup> week were recruited into the study. Some participants were excluded for reasons including induced abortions, infection, and systemic diseases such as diabetes, thyroid disease, lupus, and

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**DOI:**  
10.4103/ijpvm.IJPVM\_240\_16

### Quick Response Code:



**How to cite this article:** Kardi MT, Yousefian E, Allahveisi A, Alaei S. Association of factor V Leiden and prothrombin G20210A polymorphisms in women with recurrent pregnancy loss in Isfahan province, Iran. *Int J Prev Med* 2018;9:13.

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chromosomal abnormalities as well as uterine structural abnormalities. The control group included age-matched women who at least had one living child and no history of pregnancy loss or other gestational complications. All members of both groups were born in central Iran and were living in Isfahan Province or in areas near it.

### Molecular diagnosis

Blood samples were drawn from all women for thrombophilia testing. Factor V Leiden and prothrombin G20210 mutation were analyzed based on the reverse hybridization kits (GenID GmbH Ebinger Straberg) as previously described.<sup>[2]</sup> This process consisted of three successive steps: (1) DNA extraction from blood sample using a commercially available QIA amp DNA mini kit (Qiagen, Germany); (2) a multiplex polymerase chain reaction (PCR), thermal profile of PCR consists of initial 5 min denaturation step at 95°C, followed by 10 cycles of 95°C for 30 s, 60°C for 2 min, 22 cycles of 95°C for 10 s, 55°C for 30 s, 72°C for 30 s, and a final extension of 72°C for 8 min; (3) hybridization of amplification products to allele-specific oligonucleotide probes arrayed on test strips. Test strips revealed the wild type and mutant types. Some oligonucleotide probes were immobilized as parallel bands. Bound biotinylated sequences are observed using streptavidin alkaline phosphatase and color substrates.

### Statistical analysis

All quantitative variables were expressed in the form of mean  $\pm$  standard deviation and qualitative variables as frequency (percent). Quantitative variables compared in case and control using independent *t*-test. To compare the groups on categorical variables, Fisher's exact test was used. The odds ratio (OR) at 95% confidence interval (CI) was used to measure the standard epidemiological association between prothrombin G20210A and FVL polymorphisms in women with RPL and a control group of parous women. All analyses were performed using the Statistical Package for Social Sciences version 16 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at  $P < 0.05$ .

### Results

The population in the present study was 366 women, who were divided into two groups: Case ( $n = 250$ ) and control ( $n = 116$ ). The mean age of case group with RPL was  $29.7 \pm 3.4$  years and in the control group, it was  $30.4 \pm 3.2$  years ( $P > 0.05$ ). The mean number of pregnancy losses of case was  $2.3 \pm 0.47$ . The control group included women who at least had one living child and no history of pregnancy loss or other gestational complications. Table 1 shows characteristics of the participants. The women in the case or control groups were evaluated for FVL and prothrombin mutations. The frequencies of heterozygous FVL were 4.8% in cases and 4.3% in controls ( $P = 0.84$ ). The frequencies of homozygous mutation FVL in cases and

**Table 1: Population characteristics**

Characteristics	Case	Controls	P
Age, mean $\pm$ SD (years)	29.7 $\pm$ 3.4	30.4 $\pm$ 3.2	NS
Previous live birth	0	1-4	
Number of pregnancy losses	2.3 $\pm$ 0.47	0	

SD=Standard deviation, NS=Not significant

controls were 0.8% and 1.7% ( $P = 0.38$ ), respectively. In sum, the distribution of the various genotypes of FVL did not differ significantly among patients in case and control group [Table 2].

The frequencies of heterozygous mutation prothrombin were 6% in the case group and 0.9% in the control group ( $P = 0.025$ ). The frequencies of homozygous mutation prothrombin were 0.4% in the case group, and no mutant homozygous prothrombin carriers were identified in the control group ( $P = 0.68$ ) [Table 2].

### Discussion

The present study focused on the prevalence of FVL and prothrombin G20210A mutations in a group of Iranian women with RPL before 20<sup>th</sup> week. The analysis indicates that the prevalence of homozygous and heterozygous FVL is 1.7% and 4.3%, respectively, in healthy women. The prevalence of FVL polymorphism in our control group is similar to those observed in West Asia, in the range of 2.1%–3.8%.<sup>[7,8]</sup>

In the present study, the prevalence of heterozygous FVL mutation in the case group was 4.8%, which was higher than the control group (4.3%). Our statistical analysis found no significant association between FVL and RPL.

Some studies support our findings. Dizon-Townson *et al.* compared pregnancy-related thromboembolic events among carriers of the FVL mutation and noncarriers. They found no increased rate of RPL in carriers of the FVL mutation. As such, they concluded that if there is no history of thromboembolisms, the heterozygous carriers of the FVL mutation do not need screening or treatment during pregnancy.<sup>[9]</sup> In another study, no relationship between FVL carriers in first-trimester RPL was observed in European Caucasian females with a history of previous miscarriages.<sup>[10]</sup> Furthermore, in a similar study, researchers examined FVL mutation in 52 Japanese women with RPL and 55 parous women without any obstetric complication. They reported that there is no significant association between FVL mutation and RPL.<sup>[11]</sup> In a meta-analysis by Sergi *et al.*, they found that FVL mutation is the most common cause of inherited thrombophilia in Caucasians but is rarely encountered in African and Asian subpopulations.<sup>[12]</sup>

Some case-control studies and meta-analyses have found a high prevalence of FVL in women with RPL.<sup>[13-16]</sup> Kocher *et al.* studied 5000 pregnant women and found

**Table 2: Prevalence of factor V (FVG506A2) and prothrombin (G20210A) in women with recurrent pregnancy loss and normal controls**

Genotype	Case (n=250)	Control (n=116)	OR (95% CI)	P
<b>FVG506A2</b>				
Homozygous (GG)	94.4% (236)	94.0% (109)		0.87
Heterozygous (GA)	4.8% (12)	4.3% (5)	1.11 (0.381-3.22)	0.84
Homozygous (AA)	0.8% (2)	1.7% (2)	0.46 (0.064-3.322)	0.38
<b>G20210A</b>				
Homozygous (GG)	93.6% (234)	99.1% (115)		0.02
Heterozygous (GA)	6% (15)	0.9% (1)	8.81 (1.16-66.62)	0.025
Homozygous (AA)	0.4% (1)	0.00%		0.68

OR=Odds ratio, CI=Confidence interval

a significant association between FVL mutations and stillbirths (OR = 19.9; 95% CI: 2.07–56.94), but they did not find a similar association between FVL and early fetal loss (OR = 1.76; 95% CI: 0.85–3.65),<sup>[17]</sup> which is consistent with our results.

Our findings demonstrated that a significant association between prothrombin G20210A mutation with RPL is in agreement with another study.<sup>[3]</sup> Homozygous and heterozygous prothrombin G20210A were approximately 0% and 0.9%, respectively, in healthy females. The prevalence of prothrombin G20210A in our control group is similar to that observed in West Asia, 0.0%–1.8%.<sup>[8,18]</sup>

The prevalence of heterozygous prothrombin G20210A (GA) in women with RPL (6%) is significantly higher than the control group (0.9%). This finding is similar to several previous studies reporting significantly increased frequency of prothrombin G20210A alleles among women with RPL, including Foka *et al.*, who found that 9% of RPL patients were heterozygous for this mutation as compared to 2% in control groups.<sup>[19]</sup> In addition, Poort *et al.* reported that heterozygous carriers of the prothrombin mutation have a 2–8-fold increased risk of venous thrombosis.<sup>[20]</sup>

In a meta-analysis by Rey *et al.*, there was a significant association between prothrombin G20210A polymorphism and RPL (OR = 2.56, 95% CI: 1.04–6.29).<sup>[13]</sup> Furthermore, collaborated results from some studies revealed a strong association between prothrombin mutation and RPL.<sup>[21,22]</sup>

These analyses support our findings. However, some investigations could not find any association between prothrombin G20210A polymorphism and fetal losses in first trimester.<sup>[13,23-25]</sup> Furthermore, it is believed that in woman with thrombophilia status, a history of RPL may be associated with lower birth weight infants in subsequent successful pregnancies.<sup>[26]</sup>

Parand *et al.* conducted a case–control study of ninety patients with RPL and 44 healthy multiparous women in Southern Iran. They reported that there is no significant association between FVL and prothrombin G20210A mutation and RPL.<sup>[27]</sup> Moreover, Eskandari *et al.* found no significant differences in the prevalence of FVL and

G20210A polymorphisms between RPL and normal women.<sup>[4]</sup> The reasons for the different prevalences of prothrombin G20210A mutation in our study, compared to the above-mentioned studies, could be attributed to differences in sample sizes and ethnic backgrounds.

Furthermore, RPL is a multifactorial complication, and some of these risk factors may be insufficient for causing to RPL. The interaction between genetic variants and environmental factors increase risk of RPL.<sup>[28]</sup>

One strong point of our study was that we employed large sample sizes and reverse hybridization technique to screen for the genotypic profile of the prothrombin and FVL mutations.

## Conclusions

RPL is a challenge for gynecologists, and so far, no certain treatment has been described for this problem. Lately, thrombophilic mutation screening was presented as a way of recognizing the reasons of RPL. Our data suggest that the prothrombin G20210A mutation, but not the FVL mutation, may be an unrecognized cause of RPL in our population. However, further comprehensive studies are needed to clarify some interpretations.

## Acknowledgment

We gratefully acknowledge Dr. Baradaran for general support.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

**Received:** 05 Jul 16 **Accepted:** 05 Mar 17

**Published:** 08 Feb 18

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